SHORT-TERM AND LONG-TERM EFFECTS OF METHAMPHETAMINE ON BIOGENIC AMINE METABOLISM IN EXTRA-STRIATAL DOPAMINERGIC NUCLEI

by

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THE UNIVERSITY OF UTAH GRADUATE SCHOOL

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ABSTRACT

The effects of multiple toxic doses of methamphetamine on catecholaminergic, serotonergic, and cholinergic metabolism in the neostriatum, nucleus accumbens, olfactory tubercle, and median eminence area of the rat brain have been investigated. Methamphetamine (15 mg/kg, s.c.) was administered every 6 hours for 5 doses. Thirty-six hours after the first of 5 doses, tyrosine hydroxylase (TH) activity as well as dopamine and norepinephrine levels were significantly decreased in the neostriatum and olfactory tubercle but were not altered in the nucleus accumbens or median eminence area. Prior to the 36-hour time point, drug treatment did significantly decrease TH activity in the neostriatum (at 3, 12, and 24 hour) and olfactory tubercle (at 24 hour) but did not change the enzyme activity in the nucleus accumbens. At 0.5, 7, 15, and 30 days after 5 doses of methamphetamine, TH activity in the neostriatum was significantly decreased by at least 38%. In the olfactory tubercle, TH activity was also significantly decreased at 0.5, 15, and 30 days but was not decreased at 7 days after the last dose. Only a slight change in TH activity occurred in the nucleus accumbens at 15 and 30 days after drug treatment. In contrast, tryptophan hydroxylase (TPH) activity was significantly decreased in the neostriatum, nucleus accumbens, and olfactory tubercle by 75% 3 hours after the first dose. TPH activity remained depressed, by at least 65%, at 30 days after the

fifth dose in all 3 areas. Methamphetamine caused a slight decrease in choline acetyltransferase activity in olfactory tubercle at the 36-hour time point; however, it did not affect cholinergic enzyme activity in the neostriatum or nucleus accumbens. This evidence suggests that the serotonergic neurons are more sensitive to the toxic effects of methamphetamine than the dopaminergic neurons, whereas the cholinergic neurons seem to be unaffected.

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INTRODUCTION

Multiple intravenous injections of high doses of methamphetamine can cause psychotoxic reactions in humans. Individual doses ranging from 100-300 mg and as high as 1000 mg taken at a frequency of every 2 hours or less for approximately 3 to 6 days have been reported (Kramer, Fischman and Littlefield, 1967). Manifestations of the psychotic behavior usually occur during the mid-portion of this dosage regimen and are described primarily as paranoid schizophrenia. The behavioral symptoms are distinguished by repetitive and compulsive behavior, hallucinations (auditory, visual and tactile), and delusions of persecution (Utena, 1966; Kramer et al., 1967; and Hofman, 1975). Amphetamine (or methamphetamine) psychosis has a duration of approximately a week. However, "residual behaviors" have been reported to exist for several months (Kramer et al., 1967) to a year after termination of drug usage (Utena, 1966) and include loss of initiative and ability to concentrate, impairment of memory, and apathy. It is noteworthy that both the paranoid schizophrenic and the methamphetamine-intoxicated individual are treated with antipsychotic drugs which are primarily dopamine antagonists (Espelin and Done, 1968; Byck, 1975).

In order to determine what neurochemical changes may occur during the paranoid schizophrenic period, as well as those occurring after cessation of the drug, our laboratory designed an animal model

where the dosage regimen was similar to that used by humans abusing methamphetamine intravenously. Koda and Gibb (1973), Buening and Gibb (1974) and Kogan, Nichols and Gibb (1976) have used this model to show that multiple toxic doses of methamphetamine cause a decrease in tyrosine hydroxylase (TH) activity, and dopamine (DA), and norepinephrine (NE) levels in the rat neostriatum. Buening and Gibb (1974) and Kogan et al. (1976) have reported that the methamphetamine-induced decrease in neostriatal TH activity and DA levels could be prevented by concurrent administration of haloperidol or chlorpromazine. With this paradigm we have shown that methamphetamine also decreases neostriatal tryptophan hydroxylase (TPH) activity but does not affect choline acetyltransferase (ChAT) or glutamic acid decarboxylase (GAD) activities (Hotchkiss, Morgan and Gibb, 1979). Furthermore, the neostriatal TH and TPH activities were still significantly decreased 30 days after the last injection (Hotchkiss et al., 1979; Hotchkiss and Gibb, 1979). This work corroborates the short-term studies of Knapp, Mandell, and Geyer (1974) and McGeer, Brewall, and McGeer (1974) who demonstrated that methamphetamine decreased neostriatal TPH activity but has no effect on ChAT activity.

A number of investigations have suggested that the mesolimbic dopaminergic nuclei may play a role in amphetamine psychosis. When dopamine, apomorphine, or d-amphetamine was injected bilaterally into the rat nucleus accumbens, an increase in locomotor activity was observed (Pijnenburg and van Rossum, 1973; Andén and Johnels, 1977; Jackson, Andén, and Dahlström, 1975). Jackson et al. (1975) con-

cluded that d-amphetamine was causing release of dopamine because the drug-induced increase in locomotor activity was prevented by α-methyl-p-tyrosine or trifluoperazine, a TH inhibitor and a dopamine antagonist, respectively. Injection of haloperidol, a dopamine antagonist, into the nucleus accumbens or unilateral lesions destroying the olfactory tubercle and part of the nucleus accumbens also blocked the amphetamine-induced hyperactivity or stereotypic behavior, respectively (Pijnenburg, Hönig and van Rossum, 1975; Yehuda and Wurtman, 1975).

Using homovanillic acid (HVA) levels for an index of dopamine turnover, Andén and Stock (1973) reported that clozapine caused an increase in HVA concentration to a greater extent in the limbic area than in the neostriatum. Zivkovic, Guidotti, Revuetta, and Costa (1975) have shown that haloperidol preferentially increased the affinity of neostriatal TH for the cofactor $DMPH_4$, whereas clozapine does so in the nucleus accumbens. These investigators also reported that dopamine turnover was differentially affected by acute treatment with haloperidol and clozapine. In concentrations which did not affect turnover in the neostriatum, clozapine caused an increase in turnover in the nucleus accumbens. Conversely, haloperidol increased the turnover rate in the neostriatum but not in the nucleus accumbens. These studies suggest that the site of action of antipsychotic drugs which have a low potential for inducing extrapyramidal side effects may be in the mesolimbic brain regions.

Kaneno, Watanabe, Toru, and Shimazono (1978) found that daily administration of chlorpromazine for 14 days increased neostriatal

dopamine-sensitive adenylate cyclase activity. However, in the nucleus accumbens plus septal nuclei tissue samples, the activity was decreased, indicating that tolerance to chlorpromazine had occurred in the neostriatum but not in the nucleus accumbens.

In this study we compared the effects of multiple toxic doses of methamphetamine on dopaminergic, noradrenergic, serotonergic, and cholinergic metabolism in the neostriatum, mesolimbic nuclei (nucleus accumbens and olfactory tubercle), and the median eminence area of the rat brain in an attempt to broaden our understanding of the neurochemical changes induced by methamphetamine abuse.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 150-250 g were housed 3 per cage in a room with controlled lighting (6 a.m. to 6 p.m., light; 6 p.m. to 6 a.m. dark) and heating (24°C). Food and water were offered ad libitum. Dosage regimens and times of animal sacrifice are described in the Figure legends.

Dissection Procedure

Following decapitation, the whole brain was immediately removed from the cranium, chilled on ice and dissected on an ice-cold glass plate. With the ventral side up, 3 serial coronal sections of whole brain were made according to the following König and Klippel atlas coordinates (rostral to caudal side); 1,A10005μ-A9820μ to A8620μ-A8380μ; 2, A8620μ-A8380μ to A7890μ-A7470μ; and 3, A4230μ-A4110μ to A3750μ-A3430μ. The first coronal razor cut was made at the rostral border of the olfactory tubercle and the second approximately 1.5 mm caudal. With the aid of a dissecting scope the nucleus accumbens was bilaterally dissected from the rostral side of this 1.5-mm section. The neostriatum and olfactory tubercle were bilaterally dissected from the second serial section. The lateral olfactory tracts were used as the lateral limits and a 1-mm border caudal to the ventral surface was used as a dorsal limit for the olfactory tubercle dissection. After removel of the olfactory tubercle, the remaining

tissue was cleared away and the neostriatum was collected. The median eminence was dissected from the third coronal slice. This dissection also contains arcuate nucleus and periventricular nuclei.

After dissection, the nuclei were wrapped in parafilm, frozen immediately on solid CO_2 and stored at -80°C until assayed. This procedure, from decapitation to freezing of the tissue samples, took 4 minutes. The average tissue weights (10% mg protein/mg wet weight) were: neostriatum, 24.3 mg; nucleus accumbens, 1.61 mg; olfactory tubercle, 3.64 mg; and median eminence area, 0.53 mg. Protein measurements were determined according to the method of Lowry (1951). Preparatory procedures prior to enzymatic incubation, including reagents, were performed at 0-5°C.

Tyrosine Hydroxylase Assay

The neostriatum was homogenized in 600 µl, and the nucleus accumbens and olfactory tubercle were homogenized in 250 µl of cold 0.2% Triton X-100 in Kontes teflon-glass homogenizers (volume capacity, 1.0 ml). The median eminence from 3-4 animals were pooled and homogenized in 100 µl of 0.2% cold Triton X-100 in Kontes ground-glass homogenizers (volume capacity, 500 µl). Aliquots were taken for protein determination and the homogenates were then centrifuged at 19,000 x g at 4°C after which the supernates were assayed for TH activity by the Nagatsu method (Nagatsu, Levitt, and Udenfriend, 1964). In this procedure, L-[3,5-^{3}H] tyrosine was converted to either the L-[5-^{3}H] or L-[3-^{3}H]dihydroxyphenylalanine and the displaced tritium which equilibrates with water was counted by liquid

scintillation spectroscopy. The reaction was linear up to 15 minutes. Purification of L-[3,5-^{3}H] tyrosine was performed according to Nagatsu (1973).

The total volume (100 µl for neostriatum, nucleus accumbens, and olfactory tubercle) of the reaction medium contained: 0.4µCi L-[3, 5-^{3}H] tyrosine (Amersham) dried under nitrogen gas; 10 nmoles L-tyrosine; 100 nmoles ferrous ammonium sulfate; 200 nmoles 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine, $DMPH_4$ (Cal Biochem); 10 µmoles 2-mercaptoethanol; 20 µmoles sodium acetate; and 50 µl of tissue supernatant or 0.2% Triton X-100 for blanks. TH activity in the median eminence was assayed in a total volume of 50 µl and the reagent concentrations, as well as the volume of supernatant, were one-half that described above. The final pH of the reaction medium at 37°C was 6.0.

The reaction medium was incubated for 15 minutes in a metabolic shaker at 250 rpm at 37°C, after which the enzyme reaction was terminated with 100 µl of 10% trichloroacetic acid (TCA). The acidified reaction mixture was transferred to a Dowex 50-H^+ (200-400 mesh) column (0.5 x 2 cm). Each tube was rinsed with 1.0 ml glass-distilled water and transferred to the appropriate column after which an additional 0.8 ml of glass-distilled water was added to the column. The combined total effluent of 2.0 ml was collected in a liquid scintillation vial to which 15 ml of scintillation fluid was added. Twenty-three grams of 2,5-diphenyloxazol (PPO), 1.0 liter of Triton X-100, and 2.0 liters of toluene made up the scintillation fluid. Radioactivity was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer with an efficiency of 32%.

Tryptophan Hydroxylase Assay

The neostriatum and mesolimbic nuclei were homogenized in 100 µl and 35 µl respectively, of 50 mM HEPES buffer (Sigma) containing 5 mM dithiothreotol. Kontes teflon-glass homogenizers (volume capacity, 1.0 ml) were used for the homogenization of neostriatal tissue. Rodnoti ground-glass homogenizers (volume capacity of 100 µl) were used to homogenize pooled (2-3) mesolimbic nuclei. The homogenates were centrifuged for 15 minutes at 19,000 x g at 4°C. TPH was assayed in the supernatant by a modification of the procedure of Ichiyama, Nakamura, Nishizuka, and Hayaishi (1970), as described by Sitaram and Lees (1978), in which $^{14}CO_2$ is released from the radioactive substrate tryptophan by L-aromatic amino acid decarboxylase (L-AAAD) and trapped on Hyamine hydroxide (Sigma) soaked filter paper. The reaction was linear for at least 45 minutes.

The total volume of the reaction mixture (12.5 µl) contained: 13.3 nCi L-(1-^{14}C)-tryptophan, dried under nitrogen gas; 1.25 nmoles L-(1-^{12}C)-tryptophan; 240 nmoles HEPES buffer pH 7.4 at 25°C; 0.5 nmoles pyridoxal phosphate; 10 nmoles 6MPH_4 (absent in blanks) diluted 2:1 with a HEPES buffer (48 mM, pH 8.1) containing 480 mM mercaptoethanol; 2.5 µl (diluted 1:7.33) hog kidney L-AAAD, (partially purified by the method of Waymire, Buir, and Weiner, 1971) and 5 µl of the tissue supernatant. The final pH was 7.4 at 37°C.

The incubation of the reaction mixture and trapping of evolved CO_2 was carried out in two separate tubes connected with a small piece of tightly fitting rubber tubing. Both vessels were of the same size, 6 x 50 mm test tubes. The reaction vessels were silanized.

In addition, the trapping vessels contained a piece of filter paper, 8 x 15 mm, to which 40 µl of Hyamine hydroxide had been added. During incubation, the tubes were held together in a vertical position with a small rubber band.

Incubation of the reaction medium was at 37°C for 30 minutes, after which TPH activity was terminated by the addition of 100 µl of 5N H_2SO_4 through the rubber tubing into the reaction vessel. The rubber bands were removed and the tubes were incubated in a horizontal position at 37°C for 90 minutes during which time completion of CO_2 trapping occurred. The trapping incubation was terminated by separating the reaction vessel from the trapping vessel and transferring the filter paper to a Biovial (Beckman). The trapping vessel was rinsed with 3.5 ml of liquid scintillation cocktail (5 g PPO and 0.1 g 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, POPOP, per 1.0 liter toluene) and the combined rinse and filter paper were counted by liquid scintillation spectroscopy with an efficiency of 85%.

Choline Acetyltransferase Assay

Choline acetyltransferase (ChAT) was measured by the procedure of Fonnum (1974). The tissue samples were homogenized 1:20 with 10 mM EDTA, pH 7.4 in Kontes teflon-glass homogenizers (volume capacity, 1.0 ml). An equal volume of 1.0% Triton X-100 was added to the homogenates to complete the solubilization of the enzyme.

The total reaction mixture volume of 7.0 µl contained: 2100 nmoles NaCl, 350 nmoles sodium phosphate buffer pH 7.4, 0.7 nmoles

physostigmine (Sigma), 140 nmoles disodium ethylene diamine tetra-acetate, 56 nmoles choline chloride, 0.01 μCi [^{3}H]-acetyl-CoA (New England Nuclear), 1.4 nmoles acetyl-COA (Schwarz Mann) and 2 μl of homogenate or 2 μl of homogenizing solution for blanks. The reaction was linear for 20 minutes at 37°C and was carried out in a conical microtube, 5 x 20 mm. The reaction mixture was incubated at 37°C for 15 minutes after which ChAT activity was terminated by placing on ice and rinsing the microtube with 4.5 ml of a 10 mM sodium phosphate buffer, pH 7.4, into a liquid scintillation vial in which 2.0 ml of an acetonitrite solution containing 0.5% kalignost (Sigma) and 9.0 ml of liquid scintillation cocktail (5 g PPO, 0.1g POPOP per liter of toluene) had previously been dispensed. The phosphate buffer was added with a repipetor with enough vigor to provide for sufficient mixing of the reaction medium with the organic solvent phase. The radiolabeled product, acetylcholine, was extracted into the scintillation phase by liquid cation exchange with kalignost, whereas the substrate, [^{3}H]-acetyl-CoA, was partitioned into the aqueous phase. Since the aqueous phase contained no scintillation solvent the unconverted substrate did not interfere with the scintillation counting of the product. The scintillation vials were placed in a LS 9000 Beckman scintillation spectrophotometer and counted after the samples had cooled and phase separation was completed (approximately 10 minutes). Counting efficiency was 48%.

Dopamine and Norepinephrine Assay

Dopamine (DA) and norepinephrine (NE) were determined by a modi-

fied radioenzymatic assay of Umezu and Moore (1979) as suggested by K.E. Moore (personal communication) in which the respective substrates were methylated by partial purified catechol O-methyltransferase and S-adenosyl-L-[methyl-^{3}H] methionine to 3-methoxytyramine and normetanephrine. The tritiated methylated products were then isolated by organic solvent extraction and separated by thin-layer chromatography.

The neostriatum, nucleus accumbens, and olfactory tubercle were homogenized in 600, 100, and 150 µl, respectively, of ice cold 0.2 N percholoric acid containing 10 mg% ethylene glycol bis (β-aminoethyl-ether)-N,N'-tetraacetic acid (EGTA). The homogenates were centrifuged at 5000 x g for 15 minutes at 4°C.

The final reaction medium (35 µl) contained: 1.15 µCi S-adenosyl-L-[methyl-^{3}H]methionine (New England Nuclear); 7.6 µl catechol O-methyltransferase, purified according to a modified procedure of Axelrod and Tomchick (1958) as described by Da Prada and Zürcher (1976); 45.7 nmoles sodium ethyleneglycol-bis-(β-aminoethyl ether)N, N'-tetraacetic acid (EGTA), pH 7.2; 6.2 nmoles pargyline HCl, in 10% mercaptoethanol, freshly prepared; 9.8 nmoles Tris base containing 29.6 nmoles $MgCl_2$, pH 10.4 and 10 µl of tissue extracts, standards or 0.2 N percholic acid containing 10 mg% EGTA for blanks. Standard curves ranged from 0.05-4 ng for DA and 0.005-0.4 ng for NE (free-base, compounds obtained from Sigma). The reaction and organic solvent extraction were performed in 10 x 75 nm glass disposable culture tubes. The mixture was incubated at 37°C for 60 minutes. The reaction was terminated by placing the reaction tubes on ice, adding

30 µl of a 5:1 solution containing: 0.45 M borate buffer, pH 10 and a carrier solution containing 3-methoxytyramine and normetanephrine (5 mg/ml, free base, obtained from Sigma) and vortexing immediately. Five hundred and fifty µl of a toluene:isopentyl alcohol (3:2) solution was dispensed into each tube, vortexed and centrifuged to separate the aqueous and organic phases. Four hundred µl of the organic phase was then transferred to 40 µl of ice cold 0.1 N HCl in disposable glass culture tubes (10 x 75 mm), vortexed and centrifuged after which the aqueous phase was frozen in an acetone dry ice mixture and the organic phase was aspirated off. Twenty-five µl of the aqueous phase was then spotted on channeled Whatman LK5DF plates, allowed to dry for 1 hour and then developed in methylamine, 40%:ethanol, 100%: chloroform, 100% (5:18:40) for 80 minutes. The chromatographs were allowed to dry overnight, during which time the 3-methoxytyramine spot (R_f=0.93) and the normetanephrine spot (R_f=0.77) became visible. UV light (254 nm) was also used to visualize the spots. The spots were then marked, scraped into liquid scintillation vials and the compounds were extracted from the silica with 0.5 ml of acetic acid: ethylacetate:(H_2O) (3:3:1) for 30 minutes. Ten ml of scintillation cocktail (toluene:ethanol (95%), 7:3, containing 0.5% PPO) was added to the vials and counted in a Beckman LS 9000 scintillation counter at an efficiency of 38%. Internal standards were not run routinely, because addition of either DA or NE to neostriatal extracts showed no significant difference in dpm than that seen in perchloric acid and 10 mg% EGTA alone.

RESULTS

Short-term Effects of Methamphetamine

Figure 1 illustrates the effects of multiple toxic doses of methamphetamine (15 mg/kg, s.c., every 6 hours for 5 doses) on TH activity at 36 hours. As shown previously (Koda and Gibb, 1973; Buening and Gibb, 1974; and Kogan et al., 1976), methamphetamine induced a significant decrease in neostriatal TH activity (to 59% of control). In contrast, there was a slight decrease or no effect in the other nuclei in which TH activity was assayed. Enzymatic activity in the olfactory tubercle was significantly depressed from 14.8 to 12.7 nmoles tyrosine oxidized/mg protein/hr (a 14% decrease). However, methamphetamine induced no change in TH activity in either the nucleus accumbens or the median eminence area.

The differential decreases in TH activity correlated with NE and DA concentrations after the same drug treatment (Figures 2 and 3). Neostriatal NE and DA were significantly decreased to 32% and 18% of control, respectively. Catecholamine levels in the olfactory tubercle were also reduced (NE to 64% of control; DA to 47% of control). In the nucleus accumbens, however, the respective neurotransmitter concentrations were not significantly altered.

In order to determine whether methamphetamine caused an effect on mesolimbic TH activity prior to 36 hours, time-response relationships were determined. Figure 4 indicates that there was a time-de-

FIG. 1 Effect of methamphetamine (METH) on TH activity in the neostriatum, nucleus accumbens, olfactory tubercle and median eminence area. Rats were injected with either normal saline (1 mg/kg, s.c.; ☐) or METH (15 mg/kg, s.c.; ⚄) every 6 hours for 5 doses and decapitated 36 hours after the first dose. Numbers inside bars refer to the number of animals per group. Brackets indicate ± S.E.M.

(*) $p<0.05$ compared to saline control

(**) $p<0.001$ compared to saline control

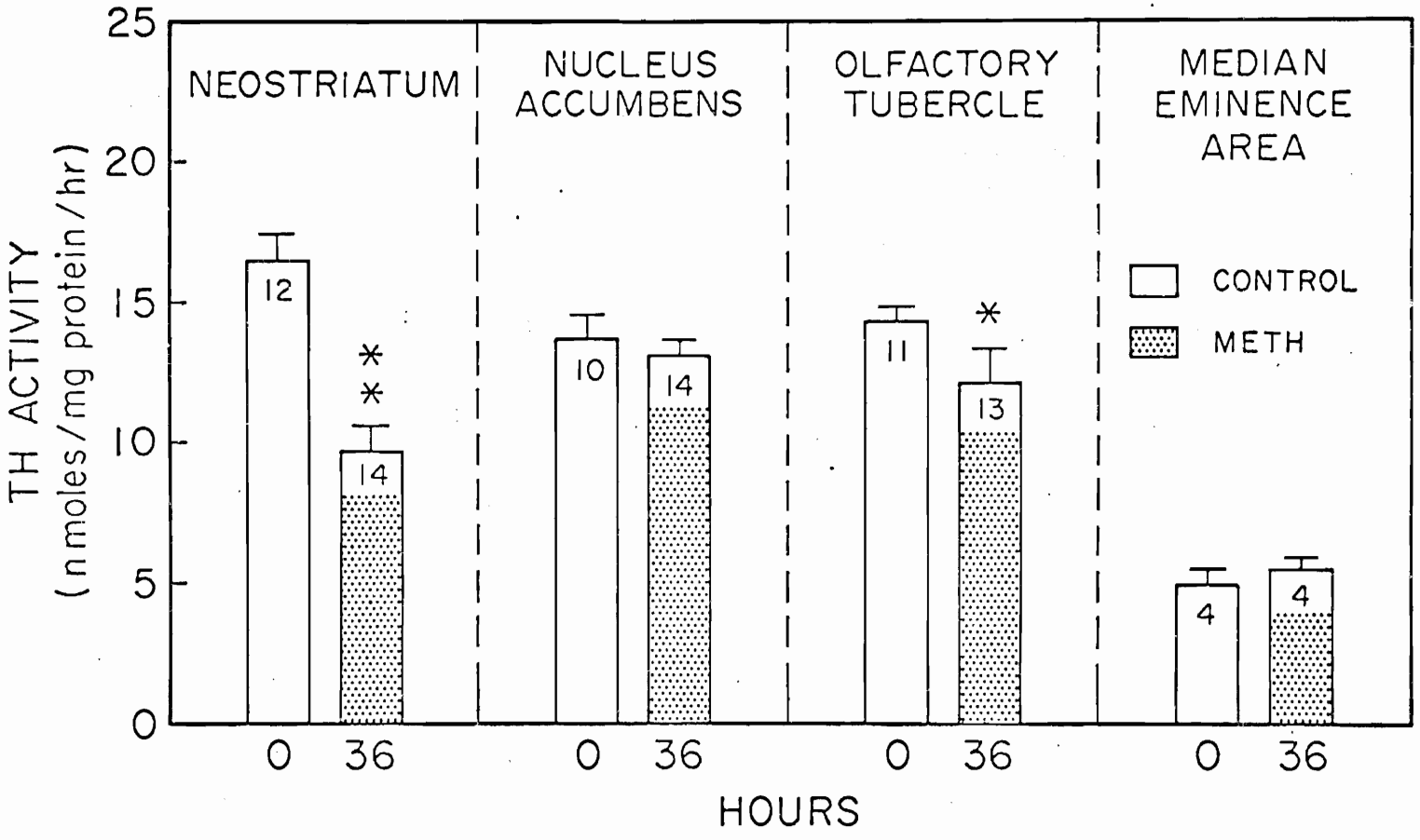

25
20
15
10
5
0
TH ACTIVITY
(nmoles/mg protein/hr)
NEOSTRIATUM
NUCLEUS
ACCUMBENS
OLFACTORY
TUBERCLE
MEDIAN
EMINENCE
AREA
CONTROL
METH
12
14
10
14
11
13
4
4
*
*
*
0
36
0
36
0
36
0
36
HOURS

FIG. 2 Effect of METH on NE levels in the neostriatum, nucleus accumbens and olfactory tubercle. Dosage regimen and decapitation time are as described in the legend of Figure 1. Numbers inside bars refer to the number of animals per group. Brackets indicate ± S.E.M.

(*) $p<0.001$ compared to saline control

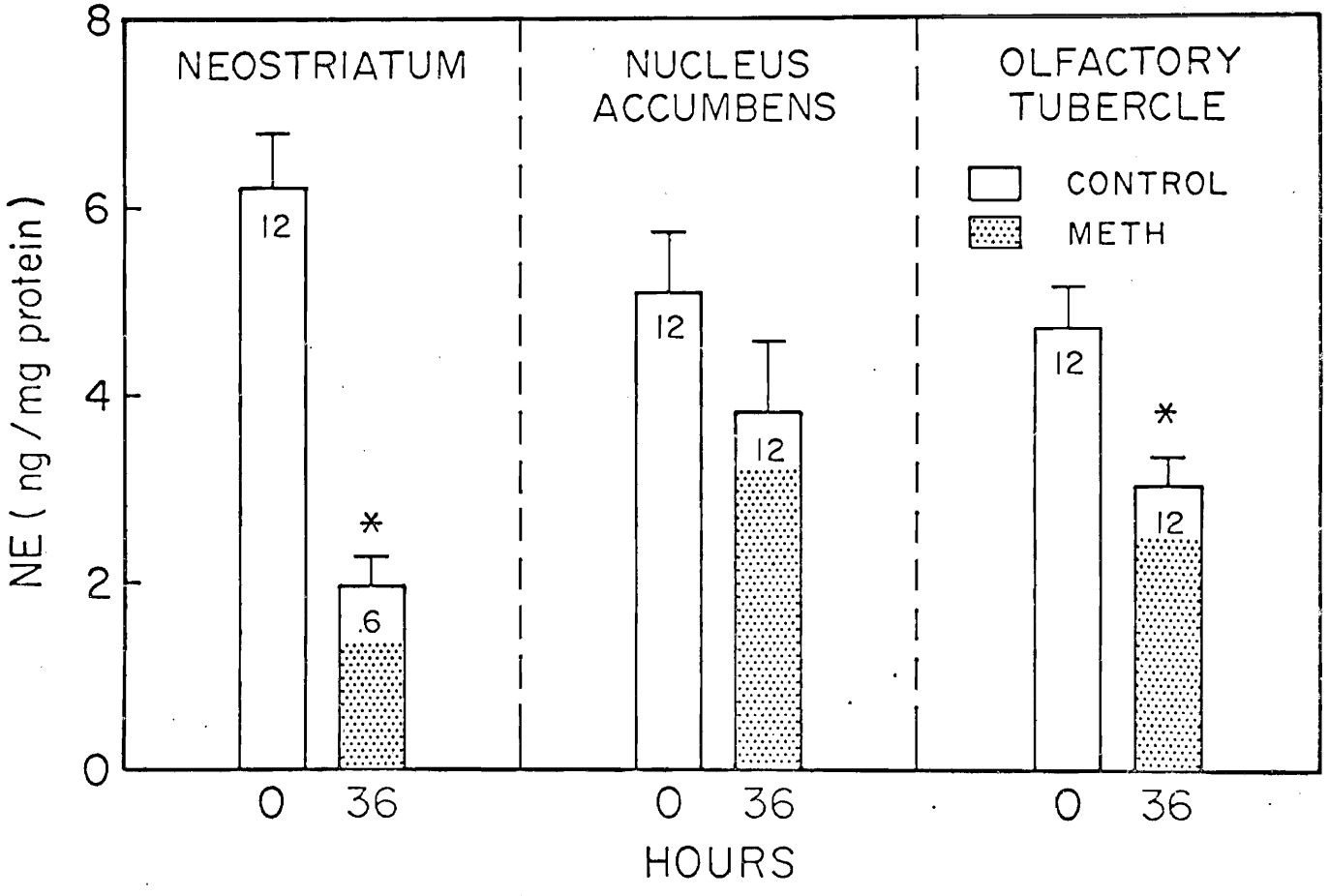

NEOSTRIATUM
NUCLEUS ACCUMBENS
OLFACTORY TUBERCLE
CONTROL
METH
NE (ng / mg protein)
8
6
4
2
0
12
12
12
12
12
.6
*
*
0
36
0
36
0
36
HOURS

FIG. 3 Effect of METH on DA levels in the neostriatum, nucleus accumbens and olfactory tubercle. Dosage regimen and decapitation time are as described in the legend of Figure 1. Numbers inside bars refer to the number of animals per group. Brackets indicate ± S.E.M.

(*) $p<0.001$ compared to saline control

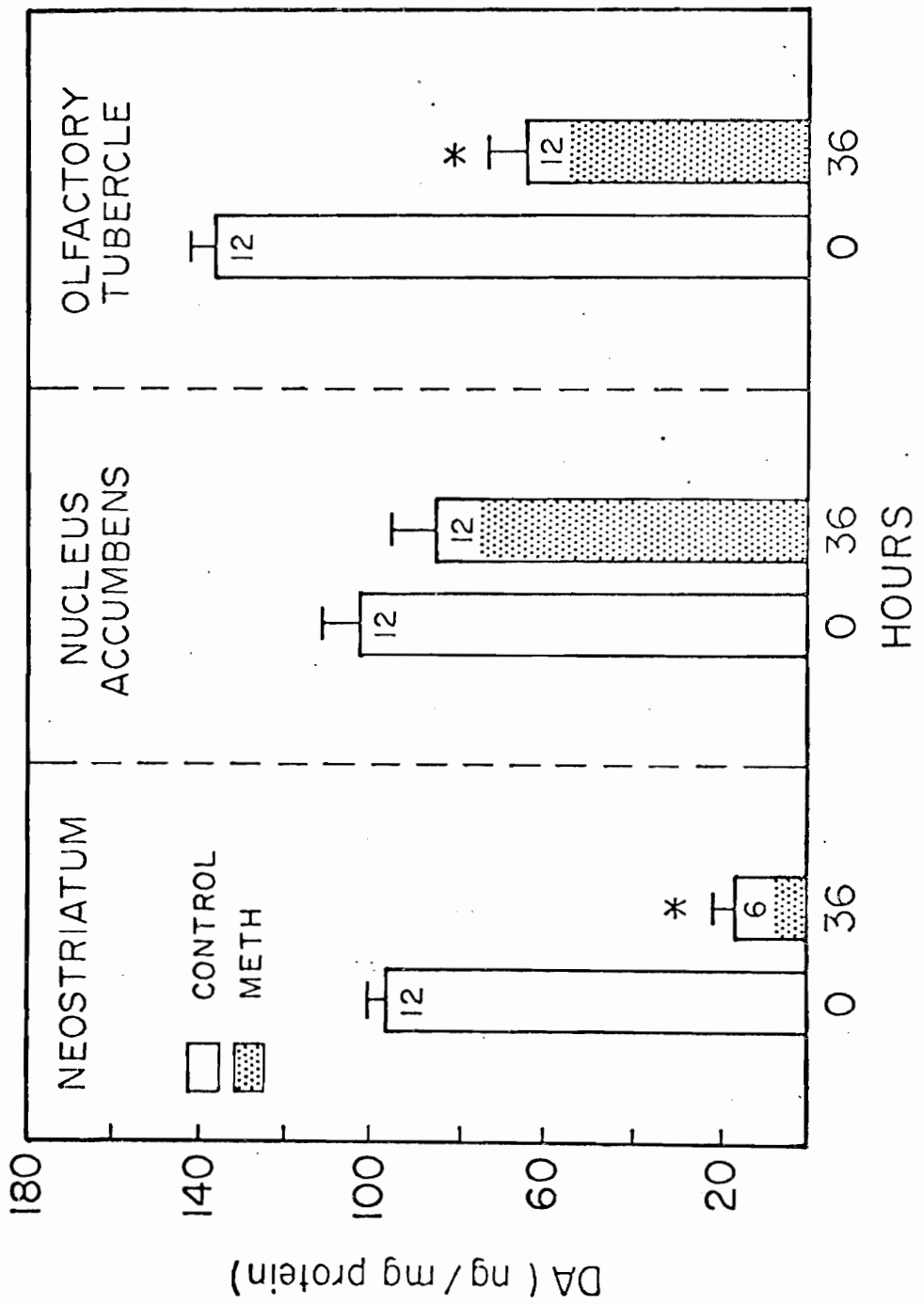
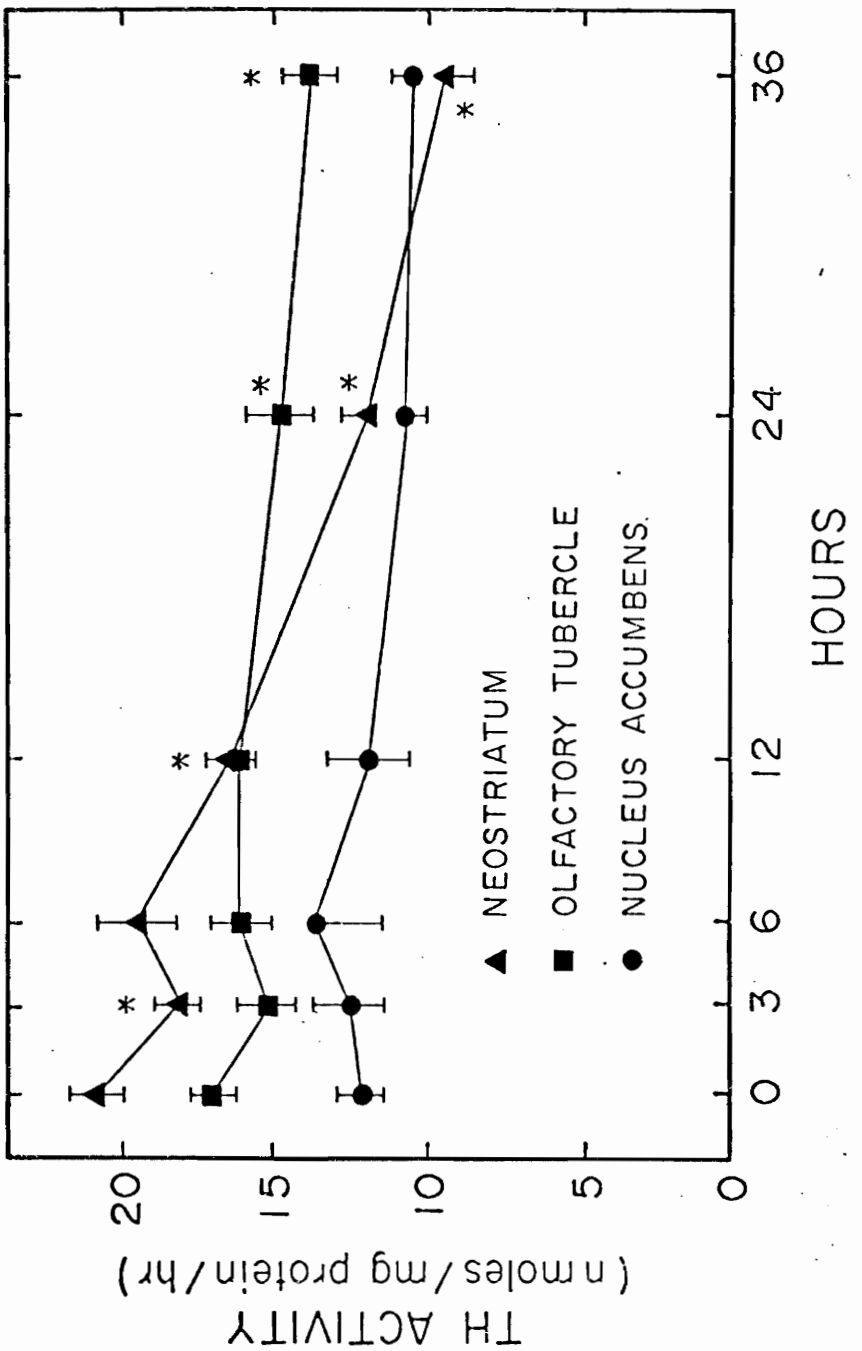

NEOSTRIATUM
NUCLEUS ACCUMBENS
OLFACTORY TUBERCLE
CONTROL
METH
DA (ng / mg protein)
180
140
100
60
20
0
36
HOURS
12
6
*

FIG. 4 Time-dependent effect of METH on TH activity in the neostriatum (▲), olfactory tubercle (■) and nucleus accumbens (●). Rats were injected with either normal saline (1 mg/kg, s.c.) or METH (15 mg/kg, s.c.) every 6 hours. To four groups of rats the following number of doses were administered: 1, 2, 3 or 5. Control and treated animals were sacrificed at 3 and 6, 12, 24 and 36 hours, respectively. The zero time point of each line represents the mean of 11-18 control observations. All other points represent the mean of 8-16 observations. Brackets indicate ± S.E.M.

(*)$<p$ 0.01 compared to saline control


TH ACTIVITY
(n moles/mg protein/hr)
0
5
10
15
20
0
3
6
12
24
36
HOURS
NEOSTRIATUM
OLFACTORY TUBERCLE
NUCLEUS ACCUMBENS.
*

pendent effect and provides additional support for a differential response to methamphetamine administration. TH activity in the olfactory tubercle was depressed significantly at the 24-hour and 36-hour time points by 12% and 18%, respectively. In the nucleus accumbens, TH activity was not significantly different from control at 3, 6, 12, 24 or 36 hours. In contrast, neostriatal TH activity was decreased more extensively and at earlier periods than that observed in the mesolimbic nuclei to 87, 79, 58 and 46% of control at 3, 12, 24 and 36 hours, respectively.

Recent investigations in our laboratory have shown that methamphetamine decreases TPH activity, but does not affect ChAT activity in the neostriatum (Hotchkiss et al., 1979). These studies have been expanded to include the nucleus accumbens and olfactory tubercle. Figure 5 shows time-dependent responses of ChAT, TH and TPH activities to 5 doses of methamphetamine in the neostriatum, nucleus accumbens and olfactory tubercle. Table 1 contains the control values used to convert the enzyme specific activities to percent control. It should be noted that the TH data in Figure 5 is that presented in Figure 4, but has been converted to percent control for comparative purposes.

The only significant alteration in ChAT activity occurred in the olfactory tubercle (a 9% decrease) at 36 hours. There was no difference in ChAT activity compared to control in the nucleus accumbens or neostriatum at the 3, 6 or 36-hour points.

The most significant effect occurred with the serotonergic neuronal enzyme marker, TPH. In all brain regions assayed and at all

Table 1

Control Values used for the Percent Control Comparisons in Figure 5

Enzyme	Hours*	Control Values †		
		Neostriatum	Nucleus Accumbens	Olfactory Tubercle
ChAT	3	137 ± 5 (15)	129 ± 7 (15)	121 ± 6 (16)
	6, 36	121 ± 5 (15)	147 ± 5 (15)	142 ± 5 (15)
TH	3-36	21 ± 1 (18)	12 ± 1 (12)	17 ± 1 (11)
TPH	3-36	0.26 ± 0.015 (30)	0.21 ± 0.015 (15)	0.21 ± 0.017 (15)

The n values for TPH in the mesolimbic nuclei represent 2-3 pooled samples. See legend of Figure 4 for decapitation schedule.

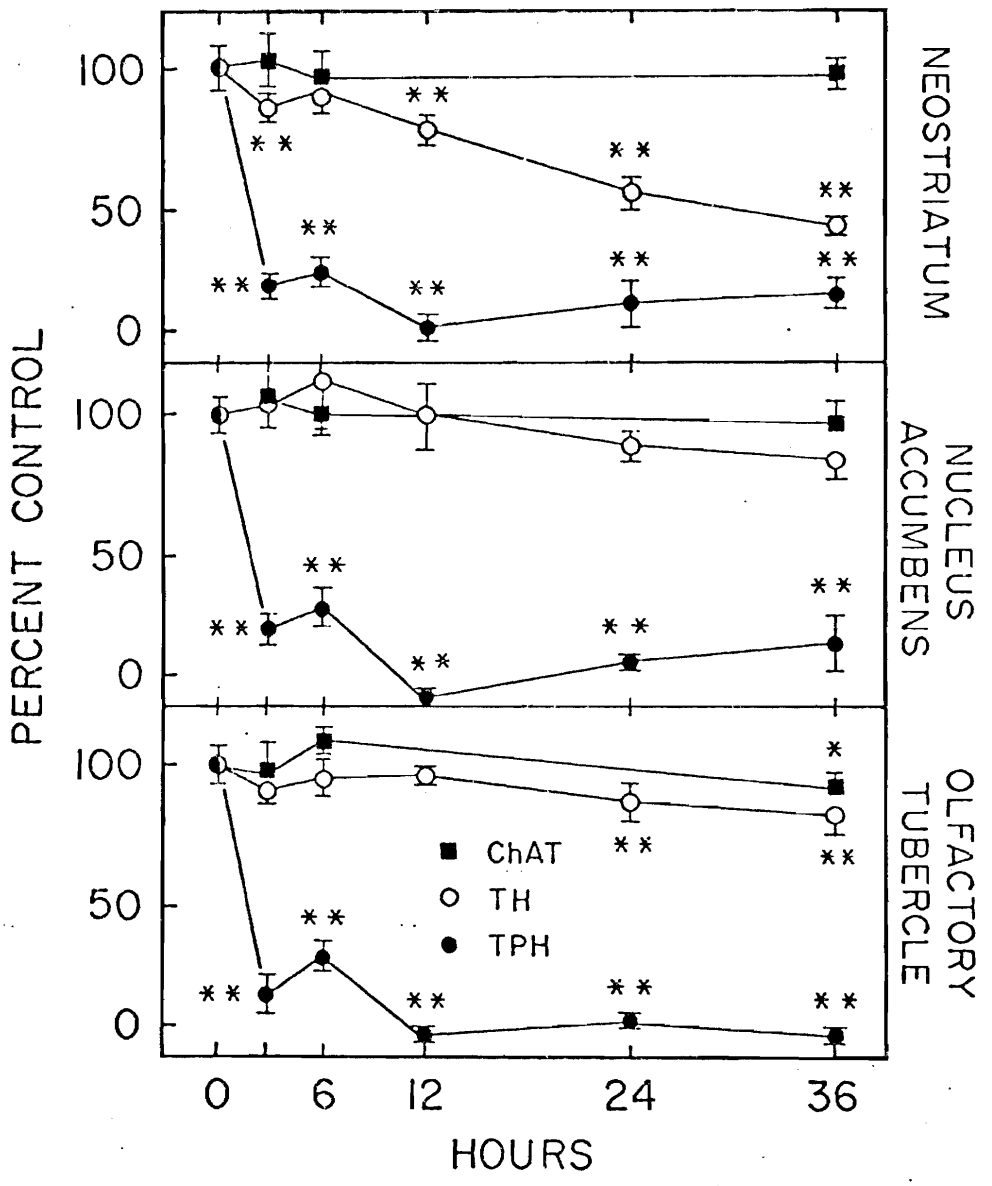
* Control values, unless otherwise indicated, were not significantly different at 3, 6, 12, 24 and 36 hour and were, therefore, accumulated.

† nmoles/mg protein/hr ± S.E.M. (n).

FIG. 5 Comparison of the time-dependent effect of METH on ChAT activity (■), TH activity (○) and TPH activity (●) in the neostriatum, nucleus accumbens and olfactory tubercle. Rats were injected with either normal saline (1 mg/kg, s.c.) or METH (15 mg/kg, s.c.) every 6 hours. See legend of Figure 4 for decapitation schedule and Table 1 for control values used for the percent conversions. The 0-hour time point (◐) represents the control values for all 3 enzymes in each of the tissue samples assayed. The n values for ChAT and TH activities for all tissue samples are the mean of 6-17 observations. The n values for TPH in the mesolimbic nuclei represent 2-3 pooled samples and the mean of 3-6 observations. Brackets indicate ± S.E.M.

(*) $p<0.05$ compared to saline control

(**) $p<0.01$ compared to saline control


PERCENT CONTROL
100
50
0
NEOSTRIATUM
NUCLEUS ACCUMBENS
OLFACTORY TUBERCLE
ChAT
TH
TPH
0
6
12
24
36
HOURS

times considered TPH activity was depressed so greatly by methamphetamine that the dpm from the treated samples were not twice blank. Therefore, it is difficult to make quantitative inferences about how much TPH activity remains during and after drug treatment. Qualitatively, TPH activity was depressed dramatically at 3 hours after the first dose of methamphetamine and stayed depressed throughout the time course. In areas where drug treatment had very little effect on TH activity (nucleus accumbens and olfactory tubercle), TPH activity was decreased to 25% of control or lower. It is noteworthy that neostriatal TPH activity almost reached its nadir (to 27% of control) at 3 hours, whereas the greatest decrease observed in neostriatal TH activity (to 46% of control) did not occur until 36 hours.

Long-term Effects of Methamphetamine

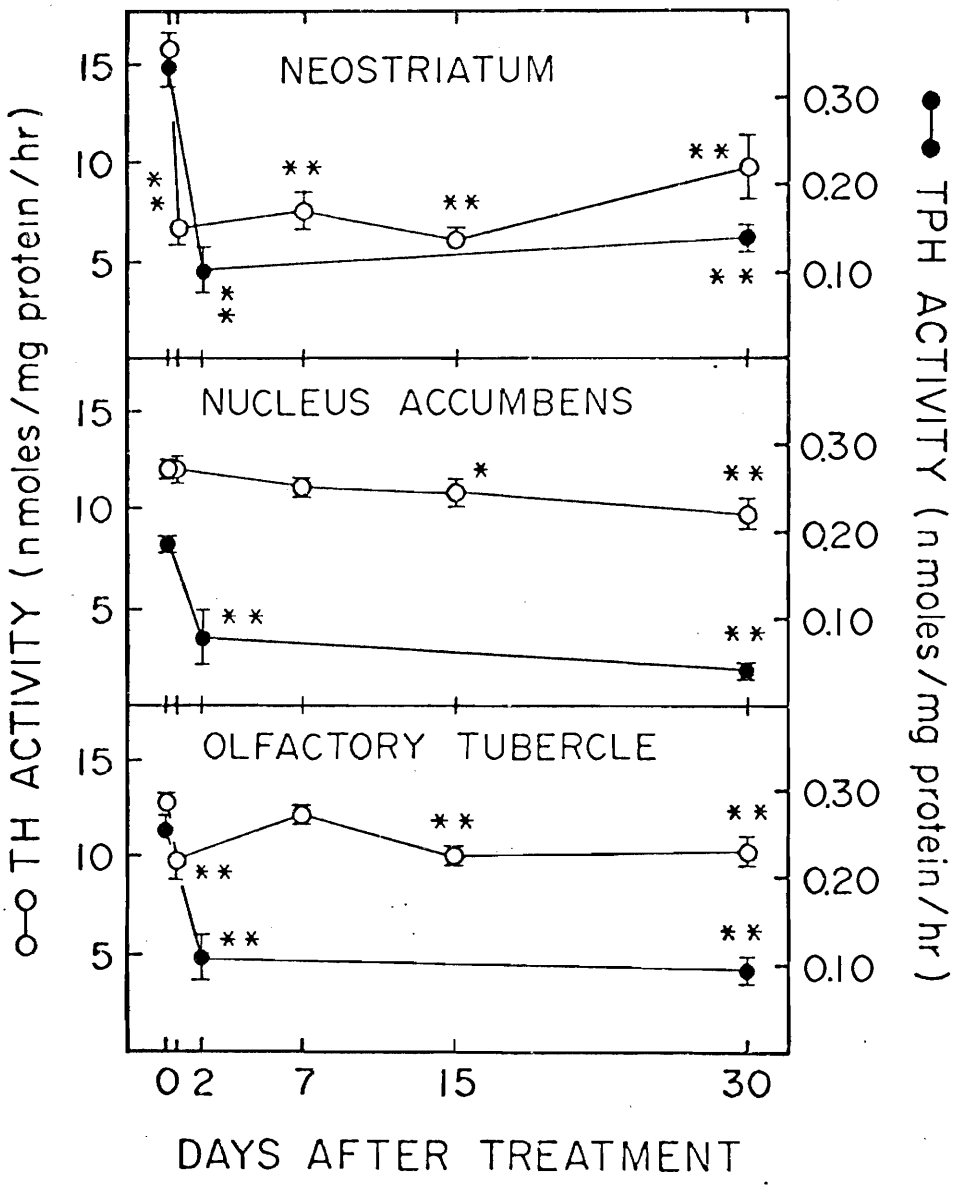
In these experiments, methamphetamine (15 mg/kg, s.c.) was administered every 6 hours for 5 doses and the TH and TPH activities were measured at various times up to 30 days after the last dose (Figure 6). The depression in TPH activity caused by methamphetamine at 36 hours (Figure 5) was still significantly decreased 2 days after the last injection in the neostriatum, nucleus accumbens and olfactory tubercle to 29, 41 and 42% of control, respectively. Thirty days after the last injection no recovery of serotonergic enzyme activity had occurred in the 3 areas assayed.

In contrast to the chronic generalized depression of TPH activity induced by methamphetamine, TH activity in various brain areas was affected differentially. Neostriatal TH activity was decreased sig-

FIG. 6 Chronic effects of METH on TH (○) and TPH (●) activity in the neostriatum, nucleus accumbens and olfactory tubercle. Rats were injected with either normal saline (1 mg/kg, s.c.) or METH (15 mg/kg, s.c.). The legend for Figure 1 describes the dosage regimen. Animals were decapitated 0.5, 2, 7, 15 and 30 days after the last dose of drug. The zero time-point of each line represents the mean of 18-27 control observations. These observations at the different time-points were not significantly different. The n values for TPH activity for the mesolimbic nuclei represent 2-3 pooled samples and were 9 for control and ranged from 2-6 for treated animals. All other points represent the mean of 6-12 observations. Brackets indicate ± S.E.M.

(*) $p<0.05$ compared to saline control

(**) $p<0.001$ compared to saline control


NEOSTRIATUM
NUCLEUS ACCUMBENS
OLFACTORY TUBERCLE
TH ACTIVITY (n moles/mg protein/hr)
TPH ACTIVITY (n moles/mg protein/hr)
15
10
5
0.30
0.20
0.10
0 2 7 15 30
DAYS AFTER TREATMENT

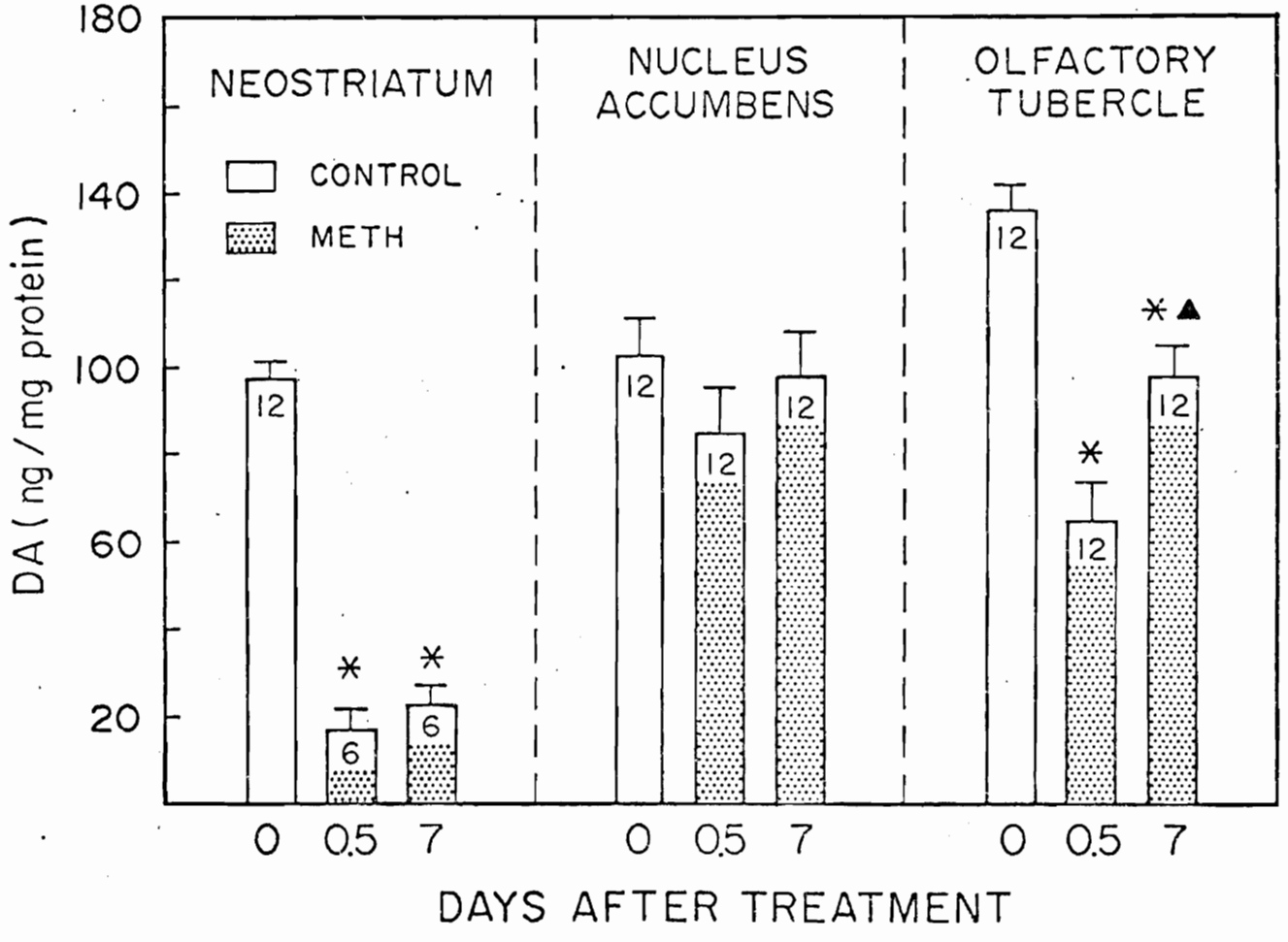
nificantly at 0.5 days and this depression persisted with little recovery for at least 30 days after the last injection. TH activity in the nucleus accumbens decreased gradually in a time-dependent manner to only 90% and 80% of control, 15 and 30 days after the last injection, respectively. In the olfactory tubercle, methamphetamine initially (0.5 day) caused a 24% depression in TH activity; however, 7 days after treatment, enzyme activity returned to control values and thereafter was significantly decreased to 78% and 79% of control at 15 and 30 days, respectively.

Dopamine values measured at 0.5 and 7 days after the last injection of methamphetamine corroborate the biphasic response observed in olfactory tubercle TH activity (Figure 7). In the olfactory tubercle, there was a significant difference between control and treated animals 0.5 and 7 days after the last injection, but the enzyme activity had increased toward normal by 7 days (25% increase compared to the 0.5-day time point, $p<0.005$). Neostriatal DA concentrations were also significantly decreased but the results did not indicate a significant biphasic response as seen in the olfactory tubercle (to 18% and 24% of control). No significant change in DA in the nucleus accumbens occurred after methamphetamine treatment.

FIG. 7 Chronic effect of METH on DA levels in the neostriatum, nucleus accumbens and olfactory tubercle. Rats were injected with either normal saline (1 mg/kg, s.c.; □) or METH (15 mg/kg, s.c.; ⊡) as described in the legend for Figure 1 and decapitated 0.5 and 7 days after the last dose of drug. Numbers inside the bars refer to the number of animals per group. Brackets indicate ± S.E.M.

(*) $p<0.001$ compared to saline control

(▲) $p<0.001$ compared to 0.5 day


180
140
100
60
20
DA (ng / mg protein)
NEOSTRIATUM
NUCLEUS ACCUMBENS
OLFACTORY TUBERCLE
CONTROL
METH
12
6
*
▲
0
0.5
7
DAYS AFTER TREATMENT

DISCUSSION

This descriptive study of the effects of multiple toxic doses of methamphetamine on biogenic amine metabolism in the central nervous system of the rat emphasizes the necessity of investigating drug-induced changes in several brain regions. Although the rat neostriatum and nucleus accumbens appear to be similar anatomically (Heimer and Wilson, 1975) and amphetamine and its congeners generally inhibit reuptake and cause release of monoamines (see Holmes and Rutledge, 1976), the present results indicate a differential effect of toxic doses of methamphetamine in these structures. More specifically, the activities of neuronal synthesizing enzymes are differentially affected during and after drug administration within individual brain regions as well as between the different areas investigated.

Comparisons of the short-term and long-term effects of methamphetamine indicate that the serotonergic neurons are more sensitive than either the dopaminergic or cholinergic neurons to the neurotoxic effects of the drug. Decreases in TH activity and in DA and NE levels were more pronounced in the neostriatum than in the mesolimbic nuclei or in the median eminence area. In fact, the catecholaminergic neurons in the nucleus accumbens and median eminence area appear to be resistant to the short-term toxic effects of methamphetamine. ChAT activity was not altered by drug treatment in the nuclei investi-

gated, which corroborates McGeer et al. (1974) who reported a lack of effect of methamphetamine on ChAT activity in the neostriatum of the rat.

A generalized effect on the serotonergic neurons did occur, however, during methamphetamine administration, i.e., TPH activity was dramatically decreased in the neostriatum, nucleus accumbens and olfactory tubercle to 27% of control or lower within 3 hours after a single dose of the drug. These data parallel the decreased conversion of tryptophan to 5-hydroxytryptamine in neostriatal synaptosomes by methamphetamine or amphetamine as reported by Knapp et al. (1974) and extend the investigations reported earlier by Hotchkiss et al. (1979).

The long-term effects of methamphetamine also provide evidence for a drug-induced differential modification of biogenic amine metabolism. TH activity in the nucleus accumbens decreased very gradually over a 30-day period. In the olfactory tubercle, a biphasic response with respect to TH activity and DA levels was observed. Neostriatal TH activity was decreased at 0.5 day and remained depressed for up to 30 days after the dosage regimen. In contrast, TPH activity was markedly depressed in all three areas for the entire 30-day time course.

The decreases observed in TH and TPH activity agree with investigations measuring the effect of chronic administration of methamphetamine or amphetamine on the levels of different neurotransmitters. Tonge (1974) administered amphetamine for 3 weeks in the drinking water of rats and found that 36 hours after cessation of

drug both NE and 5-hydroxytryptamine (5-HT) levels were decreased in several rat brain regions, including the neostriatum. Chronic administration of methamphetamine to monkeys induced a decrease in DA in the neostriatum but not in the pons-medulla, mid-brain, hypothalamus or frontal cortex 24 hours and 3-6 months post treatment (Seiden, Fischman, and Schuster, 1975). Ellison, Eison, Huberman, and Daniel (1978) administered amphetamine continuously to rats and observed a decrease in dopamine levels after 2-5 days of drug treatment and demonstrated a decrease in neostriatal TH activity 110 days after 10 days of continuous treatment. More recently, Trulson and Jacobs (1979) reported a decrease in 5-HT and DA in several regions of the cat brain 4 to 5 hours after 2 daily injections of amphetamine for 10 days.

The differential effects of methamphetamine on dopaminergic and serotonergic neurons may be explained by selective uptake of methamphetamine into the respective nerve terminals. A number of investigators have shown that amphetamine is localized in nerve terminals (Wong, Vanfrank, Horng, and Fuller, 1972; Azzoro, Ziance, and Rutledge, 1974; and Jori, Caccia, and Garattini, 1977) and that the uptake of low concentrations of ^{3}H-amphetamine into synaptosomes is blocked by two different uptake inhibitors, cocaine and desipramine (Azzoro et al., 1974). There is also evidence that the uptake system has stereoselectivity. Horn (1973) demonstrated that the noradrenergic terminal sites were more sensitive to changes in the basic β-phenethylamine structure than were the dopaminergic terminals with respect to inhibition of uptake of NE and DA into synaptosomes

of rat hypothalamus and neostriatum, respectively. Raiteri, Bertollini, Angelini, and Levi (1974), referring to Horn's work, suggested that the differential effect of amphetamine in releasing ^{3}H-monoamines from different brain regions may be due to the less structural and stereochemical specificity of the uptake sites for DA as compared to NE and that such a relationship may provide d-amphetamine greater accessibility to dopaminergic storage sites than to noradrenergic storage sites. This difference would permit a higher accumulation of amphetamine into the dopaminergic terminals. The present data provide supportive evidence for differential accessibility by comparing the effects of methamphetamine on catecholamine levels in the neostriatum and/or olfactory tubercle in which the amount of depletion is less for NE than it is for DA (neostriatum, to 32 and 18% of control, olfactory tubercle, to 64 and 47% of control; levels of NE and DA, respectively).

Perhaps the uptake system for the serotonergic nerve terminals is even less sensitive to stereochemical requirements than either of the catecholamine terminals leading to a greater accumulation of methamphetamine into serotonergic systems. Evidence that methamphetamine may be taken up into serotonergic terminals comes from recent studies indicating that the methamphetamine-induced decrease in TPH activity but not in TH activity in the neostriatum is blocked by a specific 5-HT uptake inhibitor, fluoxetine (Hotchkiss and Gibb, personal observations). However, if amphetamine concentrations are measured in the neostriatum after 6-hydroxydopamine or 5,6-dihydroxytryptamine, only the former neurotoxin decreases the drug concentra-

tion (Jori et al., 1977). These observations suggest that amphetamine is localized in catecholaminergic not serotonergic terminals in the neostriatum. Further studies are needed to clarify these results.

The lack of a methamphetamine-induced decrease on TH activity and catecholamine levels, but not on TPH activity, in the nucleus accumbens emphasizes the sensitivity of the serotonergic nerve terminals to the neurotoxic effects of this drug. Other investigators have also shown that the dopaminergic terminals in the nucleus accumbens are affected less by amphetamine treatment than are other dopaminergic areas of the brain. Kuczenski (1979) reported that amphetamine blocked a haloperidol-induced increase in rat neostriatal TH activity and DOPAC levels but no blockade occurred in the nucleus accumbens with amphetamine. K. E. Moore (personal communication) has also shown that amphetamine had no significant effect on TH activity in the nucleus accumbens.

In conclusion, we have shown that TH and TPH activities are differentially depressed during methamphetamine administration. The decrease in enzyme activity occurs more rapidly and more profoundly in the serotonergic neurons than in the dopaminergic neurons and persists for at least one month after drug treatment with little, if any, recovery of TPH activity. In light of the postulated involvement of the mesolimbic nuclei in paranoid schizophrenia (Stevens, 1975; Hornykiewicz, 1978; and Farely, et al., 1978) it is interesting to note that in this animal paradigm the most dramatic alterations induced by methamphetamine in these nuclei occurred in the sero-

tonergic nerve terminals. Inference suggests that the motor and emotional disturbances associated with amphetamine psychosis and paranoid schizophrenia may be due, in part, to a multiple disruption in the synthesis of both catecholamines and 5-HT.

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